

**MIGRATION OF GENES FROM ORGANELLES TO THE NUCLEUS:
THE CASE OF TWO GENES ENCODING PHOTOSYNTHETIC
MEMBRANE PROTEINS OF *Euglena gracilis***

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We have studied the migration of organellar genes to the nucleus in photosynthetic protists. The initial studies were centered on the migration of mitochondrial genes in chlamydomonad algae. Since the genes *cox2*, *cox3*, and *atp6* were absent in the mtDNA of *Chlamydomonas reinhardtii*, we investigated whether these genes were expressed in the nucleus. The genes *cox2a*, *cox2b*, *cox3*, *atp6* and *nad4L* - encoding subunits II and III of cytochrome *c* oxidase (COXIIA, COXIIB, and COX III), subunit ATP6 (subunit "a") an essential component of the proton translocating F₀ sector of the F₁F₀-ATP synthase, and subunit NAD4L of NADH-ubiquinone reductase (Complex I) respectively - where shown to reside in the nucleus of *C. reinhardtii*, in contrast to the mitochondrial location of these genes in the vast majority of eukaryotes [1,2]. Several specific features have accompanied the migration of genes from organelles to the nucleus: *i*) Acquisition of a targeting sequence or a transit peptide. In some cases, organellar genes have inserted into nuclear genes, acquiring the pre-existing targeting sequences. Acquisition may also occur by duplication of existing targeting signals or by nuclear exon shuffling. *ii*) Acquisition of introns, promoters, and ribosome binding sites. *iii*) Acquisition of polyadenylation signals. *iv*) Change in codon usage. Relocalization of organellar genes to the nucleus is usually followed by changes in codon usage to frequencies typically found for nuclear genes. *v*) Inactivation of the organellar gene copy, and loss of the original organellar gene. Successful migration of genes from organelles to the nucleus is usually followed by inactivation of the mitochondrial or chloroplastic copy, its conversion into a pseudogene, and its eventual loss from the organellar genome.

Migration of genes encoding highly hydrophobic components poses the question of how the corresponding proteins are imported back into their target organelle. In yeast, *in vivo* studies with cytoplasmic synthesized constructs of variable lengths of apocytochrome *b*, suggested that the highest average hydrophobicity over 60 to 80 amino acids of a polypeptide chain (*mesoH*), along with the maximum hydrophobicity of the putative transmembrane segments, are useful indicators of the likelihood that a protein could be imported into mitochondria

[3]. Accordingly, the corresponding nuclear-encoded proteins of chlamydomonad algae COX IIA, COX IIB, COX III and ATP6, all exhibit reduced overall hydrophobicity (both decreased *mesoH* and $\langle H \rangle$) that allow them to be imported into mitochondria. In addition, it was proposed that the selective decrease in the hydrophobicity of organellar proteins encoded by nuclear genomes is stronger in those transmembrane regions that seem not to be critical for function, assembly, or participation in inter-subunit interactions with other constituents of the complexes [4]. Here, we addressed the study of two chloroplast genes that migrated to the nucleus in the photosynthetic protist *Euglena gracilis*. *Euglena* contains chloroplasts surrounded by three membranes which are thought to have arisen from a secondary endosymbiotic process. The genes *petA* and *petD*, encoding cytochrome *f* (a protein with two transmembrane stretches) and subunit IV (a protein with three transmembrane helices) of the cytochrome *bf* complex are not present in the chloroplast DNA (cpDNA) of *E. gracilis*. We asked whether these genes had been relocated to the nucleus. The *Euglena bf* complex was isolated, and the presence of cytochrome *f* and subunit IV in the complex was established by Western blot analysis, by heme-specific staining after SDS-PAGE, and by N-terminal amino acid sequence analyses. Based on N-terminal and conserved internal protein sequences, oligodeoxynucleotides were designed and used for PCR gene amplification and cDNA sequencing. The complete sequence of the *petA* cDNA and the partial sequence of the *petD* cDNA from *E. gracilis* were obtained [5]. The *petA* gene encodes an atypical cytochrome *f*, with a unique insertion of 62 residues not present in plant and algal *f*-type cytochromes. The *petA* gene also acquired a region that encodes a large tripartite chloroplast transit peptide, which mediates the transfer of apocytochrome *f* through the three-membrane chloroplast of *E. gracilis*. Evidence was provided that in *Euglena* the *petA* and *petD* genes have migrated from the chloroplast to the nucleus. Both *petA* and *petD* genes exhibit a typical nuclear codon usage, clearly distinct from the usage of genes localized in the chloroplast, and exhibit diminished hydrophobicity as compared with its plant and algal chloroplast-encoded counterparts. This is the first description of *petA* and *petD* genes that are nucleus-localized. We are currently overexpressing in *Escherichia coli* a cytochrome *f* fragment lacking the transmembrane region, in an attempt to crystallize and characterize this protein.

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